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Review

Microglia: Multitasking Specialists of the Brain

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Microglia are macrophages that colonize the brain during development to establish a resident population of professional phagocytes that protect against invading pathogens and contribute to brain development and homeostasis. As such, these cells sit at the interface between immunology and neurobiology. In addition to their key roles in brain physiology, microglia offer a great opportunity to address central questions in biology relating to how migrating cells find their positions in the embryo, adopt a behavior that is appropriate for that position, and interact with their local environment. We aim, in this review, to survey key recent advances in microglial research.

Microglia are the tissue-resident macrophages of the brain, and as such, they serve both immune-related and glial functions by taking part in the development and homeostasis of this organ. Microglia have a number of key features—including their long-range migration to the brain during development, their adaptation to the local environment, and their role in brain development—that make them a great resource for addressing basic cellular and developmental questions. Having established the importance of these cells in many different physiological and pathological processes, current research on microglia is moving toward a mechanistic understanding of this multifunctional cell type. In this review, we discuss recent advances in microglial biology, with a particular focus on the early stages of brain development.

Tissue-Resident Macrophages: One Cell Type Coming in Many Flavors

Tissue-resident macrophages are found in most vertebrate organs and constitute a heterogeneous population of immune cells that share a common myeloid origin but have distinct morphologies and functions (Figure 1). For instance, we find highly branched microglia in the brain, Kupffer cells in the liver, and multinucleate osteoclasts in bones. These cells are mostly known for their central role in host defense as, during infection, they engulf foreign particles and alert the rest of the immune system. However, accumulating evidence indicates that resident macrophages also serve important developmental functions. For example, in osteopetrotic mice, characterized by low numbers of macrophages (Wiktor-Jedrzejczak et al., 1990), the development of the mammary gland is delayed and abnormal (Gouon-Evans et al., 2000), and lungs are affected by the accumulation of surfactant phospholipids and proteins (Trapnell and Whitsett, 2002). Macrophages are equally important and fulfill tissue-specific functions also in adulthood. For instance, Kupffer cells are involved in lipid metabolism and bilirubin production in the liver (for a review, see Naito et al., 2004), osteoclasts mediate matrix degradation in the bones (for reviews, see Charles and Aliprantis, 2014), and red pulp macrophages regulate erythrocyte degradation and iron recycling (Kohyama et al., 2009).

It is now clear that tissue-resident macrophages, although sharing immunological functions, have also specialized roles that depend on the organ of residence. Understanding how this is achieved is, of course, of great importance. It is conceivable that phenotypical and transcriptional differences among these cells arise after naive cells enter host organs and adapt to locally derived instructive signals. Evidence for this mechanism comes from peritoneal macrophages, where tissue-derived retinoic acid signaling regulates the transcriptional program of these cells via the expression of *Gata6* (Okabe and Medzhitov 2014; Rosas et al., 2014; Gautier et al., 2012). In line with this, transforming growth factor β (TGF-β) was shown to be critical for the acquisition of microglial molecular signatures (Butovsky et al., 2014), while Heme, the result of erythrocyte degradation, has been shown to induce *Spi-c* expression in red pulp macrophage (Haldar et al., 2014). In this context, it is central to establish how the environment impacts on these cells. Many groups have recently addressed this issue by comparing the chromatin landscape and the transcriptome of several tissue-resident macrophage populations (Kohyama et al., 2009; Gautier et al., 2012; Lavin et al., 2014; Butovsky et al., 2014). In particular, mapping enhancer histone modifications revealed that genes commonly found in all macrophages are differentially expressed within distinct populations, indicating that specific profiles and chromatin landscapes are likely to result from the crosstalk between the local microenvironment and the cell ontogeny (Heinz et al., 2010; Lavin et al., 2014; Gosselin et al., 2014). It is interesting that the instructive role of the microenvironment has been shown in adult bone marrow (BM) transplantations. Chromatin analysis of BM-derived lung, spleen, and liver macrophages revealed that, several months after transplantation, these cells modify their enhancer landscape, acquiring features similar to those of endogenous tissue-resident counterparts. It has also been shown that, remarkably, differentiated tissue-resident macrophages are plastic and can adapt to new environments. In particular, peritoneal macrophages transplanted in the lung, although retaining many peritoneal markers, upregulate lung-macrophage-specific genes, such as *Chi3l3*, *Sftpc*, and *Car4* (Lavin et al., 2014). In line with this, peritoneal macrophages cultured in vitro with interleukin (IL)-34 and colony-stimulating factor-1 (CSF1) reduce their peritoneal-specific gene expression and acquire additional microglial markers (Gosselin et al., 2014). Although these studies have offered many insights into key aspects of tissue-resident macrophages, it remains to be established if these cells differ before organ colonization.

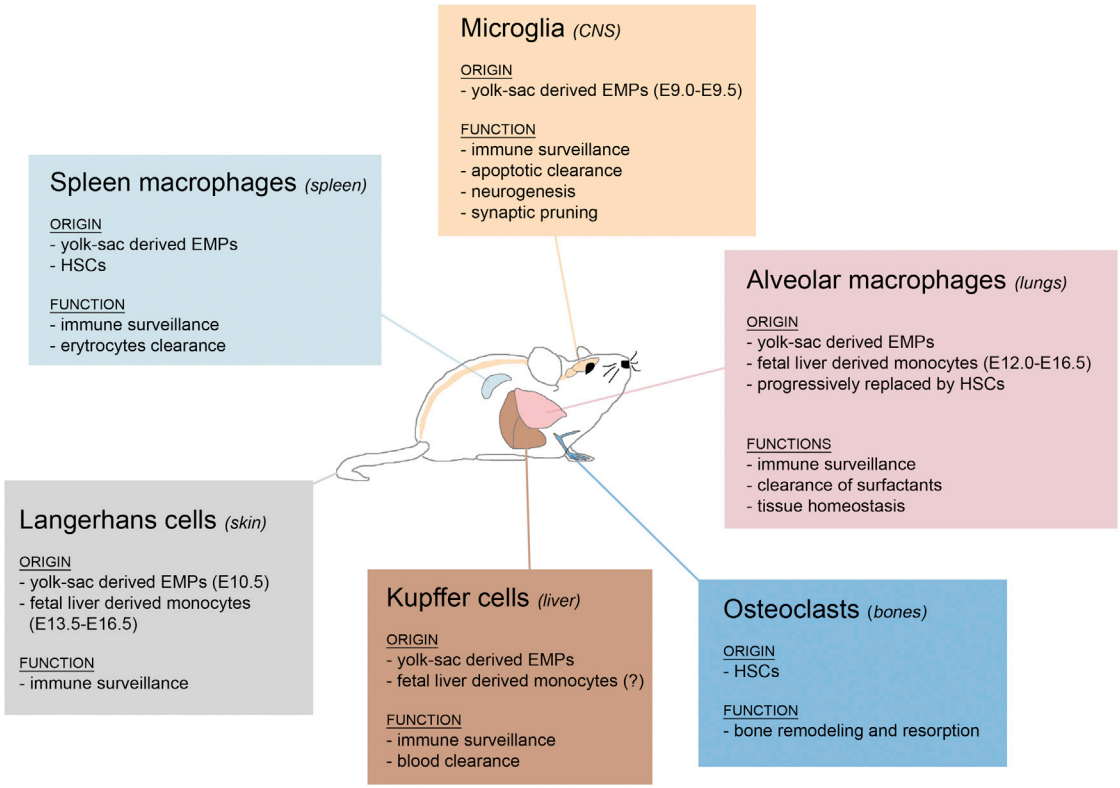

Figure 1. Heterogeneity of Tissue-Resident Macrophages

Adult macrophages are broadly distributed within organs and tissues, where they provide immune surveillance and homeostatic functions. These cells serve distinct tissue-specific roles and have multiple myeloid origins. Macrophages in the brain (microglia) originate from yolk-sac-derived EMPs and are maintained until adulthood through local self-renewal (Ginhoux et al., 2010; Perdiguero et al., 2014). Alveolar macrophages have been shown to derive from yolk-sac EMPs and fetal-liver-derived monocytes and are progressively replaced by HSCs during aging (Guilliams et al., 2013; Perdiguero et al., 2014). Recent studies have shown that yolk-sac EMPs give rise to Kupffer cells in the liver (Perdiguero et al., 2014); however, whether fetal-liver-derived monocytes are also involved cannot be completely excluded (Ginhoux and Jung, 2014). Langerhans cells have a dual origin, involving both yolk-sac EMPs (Hoeffel et al., 2012; Perdiguero et al., 2014) and fetal-liver-derived monocytes (Hoeffel et al., 2012). Macrophages in the spleen derive from HSCs (Orkin and Zon, 2008), although recent work has suggested that these originate from embryonic yolk-sac precursors (Perdiguero et al., 2014). Finally, osteoclasts derive from HSCs (Lorenzo, 2007).

Macrophages in the Brain: The How and the When

In recent years, the brain-tissue-resident macrophages have been the subject of much attention. Although long debated, it is now clear that microglia have a mesodermal origin (Rio-Hortega, 1939; Murabe and Sano, 1982). Support for this comes from the fact that, in the absence of the myeloid transcription factor PU.1 in both mice and zebrafish, there are no microglia in the brain (Beers et al., 2006; Herbomel et al., 1999). Cell lineage experiments in mouse have shown that microglia originate in the yolk sac in a *Pu.1*-dependent and *Myb*-independent manner (Ginhoux et al., 2010; Schulz et al., 2012). More recently, fluorescence-activated cell sorting approaches in mouse have identified, in erythromyeloid progenitors (EMPs), the precursors of microglia (Kierdorf et al., 2013a; Perdiguero et al., 2014). In particular, Kierdorf and colleagues have shown that $CD45^-$ and $ckit^+$ cells in the yolk sac give rise to CX3CR1, $CD45^+$-positive macrophages, and $Ter119^+$ erythrocytes in vitro (Kierdorf et al., 2013a). Recent work has also shown that tissue-resident macrophages of brain, liver, lungs, and skin share a common origin and derive from *Tie2*-expressing EMPs, a lineage that is distinct from that of hematopoietic stem cells (HSCs) (Perdiguero et al., 2014).

In the mouse, microglia begin appearing in the brain soon after production in the yolk sac, at embryonic day (E)8.0, and their migration into the brain is blood circulation dependent, as shown in *Ncx* mutants, which have no heartbeat and lack microglia (Koushik et al., 2001; Ginhoux et al., 2010). Regarding the origin of microglia, it remains to be established if there is predetermination among tissue-resident macrophages. One way to uncover this could come from the identification of factors specifically required for brain colonization. In zebrafish, embryonic macrophages are produced in large amounts at ~20 hours postfertilization (hpf) in the anterior lateral plate mesoderm and later, at 30 hpf, in a posterior region located in the trunk called the intermediate cell mass (Stachura et al., 2011; Xu et al., 2012). These cells spread within the embryo, and at 48 hpf, a few macrophages begin invading the brain, thereby establishing a microglial population that, in the embryo, is composed of ~25 cells (Herbomel et al., 2001) (Figure 2). This small number is suggestive of mechanisms that govern colonization and control the size of the initial microglial pool. It is interesting that *Drosophila melanogaster* hemocytes, the fly equivalent of the vertebrate macrophages, undergo stereotypic migration patterns to populate the developing embryo. Genetic analysis has shown that these cells are guided

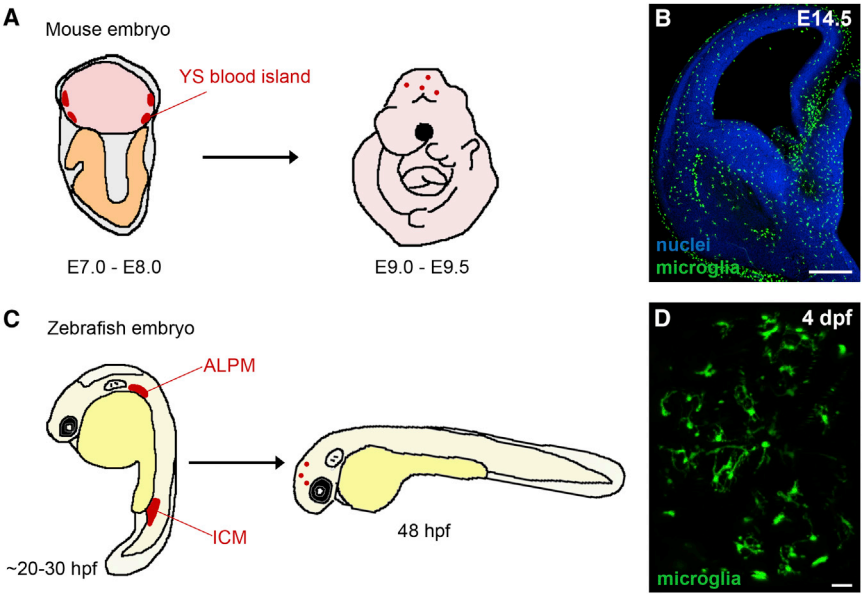

Figure 2. Embryonic Development of Microglia in Mouse and Zebrafish

(A) In mouse, primitive macrophages are generated at E7.0–E8.0 in the yolk-sac (YS) islands and colonize the brain around E9.0–E9.5, giving rise to the microglial population.

(B) Microglia distribute evenly across the encephalic tissues. Coronal section of a *Cx3cr1*$^{+/gfp}$ mouse brain at E14.5 showing GFP^{+} microglia (credit to P. Squarzoni and S. Garel). Scale bar, 500 μm.

(C) In zebrafish, macrophages originate in the anterior-lateral-plate-mesoderm (ALPM) and in the intermediate cell mass (ICM) between 20 hpf and 30 hpf, respectively. The first microglia appear in the brain around 48 hpf.

(D) Kaede^{+} microglial cells in the brain of an *mpeg1::GAL4-UAS::Kaede* zebrafish embryo 4 days postfertilization (dpf). Scale bar, 20 μm.

by the expression of PVF2 and PVF3, secreted proteins with similarity to the vertebrate platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) families. Knocking down *Pvf2* leads to a lack of hemocytes along the dorsal vessel and fewer cells at the ventral midline (for a review, see Wood and Jacinto, 2007). It is possible that equivalent developmental cues also exist in vertebrates in order to guide tissue-specific macrophages toward given organs. However, the nature of these guiding molecules is unknown. Several reports have shown that mouse embryonic macrophages express the CSF1 receptor (CSFR1) and that, in its absence, there is a significant loss of yolk sac macrophages and microglia, both in the embryo and in the adult (Ginhoux et al., 2010; Stanley et al., 1983; Cecchini et al., 1994; Elmore et al., 2014). Similarly, lack of CSF1 or of the newly identified IL-34 ligand leads to a microglial reduction in the adult brain, suggesting a role for this pathway in microglial survival and differentiation (Wang et al., 2012; Greter et al., 2012). Also, loss-of-function mutation in *fms*, the *Csfr1* zebrafish ortholog, leads to a transient loss of microglia during early development (Herbomel et al., 2001). More recently, a zebrafish genetic screen has identified the phosphate exporter *xpr1b* as a new player in microglial development. *Xpr1b*, the zebrafish ortholog of the human *XPR1*, encodes a conserved transmembrane protein that contributes to the maintenance of cell phosphate balance (Giovannini et al., 2013). Xpr1b is essential for the development of several types of tissue-resident macrophages, including microglia (Meireles et al., 2014). The same group has also identified a mutation in the *nlrc3-like* gene, causing macrophages to mount an unwanted inflammatory response. As a result, macrophages fail to follow hardwired developmental migration paths and to establish tissue-resident populations, including microglia. These studies indicate that macrophages need to regulate their activation state in order to respond to developmental cues (Shiau et al., 2013). The issue of how macrophages prioritize between different stimuli has also been addressed in *Drosophila.* Here, cell death induced with a laser can attract hemocytes that move rapidly toward the damaged area. As the wound closes, these cells return toward the ventral midline, where the expression of PVF2 and PVF3 guide their spreading across the embryo (Moreira et al., 2010).

In conclusion, work so far has led to the identification of key factors involved in microglia specification and guidance; these factors, however, are not microglia specific. The isolation of molecules that specifically drive brain colonization by microglia is of great importance and may set the groundwork for the development of means for delivery into the brain in the context of neurodegenerative diseases.

Microglia: Maintaining the Status Quo

The fact that the microglial population is established during early embryogenesis raises several questions. One, in particular, is how these cells are maintained, are turned over, and adapt to changes in brain heath status. Studies in mice using BM irradiation and chimerism have focused on understanding whether microglial maintenance relies on self-renewal or requires contribution of circulating monocytes. These approaches have shown that, under physiological conditions, circulating monocytes do not contribute to the adult microglial pool, despite high rates of colonization to other organs, such as the liver (Ajami et al., 2007; Mildner et al., 2007). This finding has also been confirmed by lineage-tracing studies in mice, indicating that self-renewal is the driving force behind microglial turnover in the adult CNS (Ginhoux et al., 2010). However, there is accumulating evidence for blood-derived-monocyte and BM-derived-cell infiltration into the CNS under pathological conditions. Studies in the experimental autoimmune encephalitis mouse model have shown that blood-derived myelomonocytes actively populate the brain, giving rise to cells that are phenotypically indistinguishable from resident microglia. However, these cells remain in the brain only transiently and do not contribute to the resident microglial pool (Ajami et al., 2011). In contrast, upon BM transplantation, engrafted cells generate mature microglia, indicating that this is limited to uncommitted hematopoietic cells (Priller et al., 2001; Ajami et al., 2011). It is interesting that induction of demyelination and neurodegeneration in mice revealed that pathology, per se, is not sufficient to trigger monocyte infiltration in the brain and that CNS conditioning, such as irradiation, is required to allow

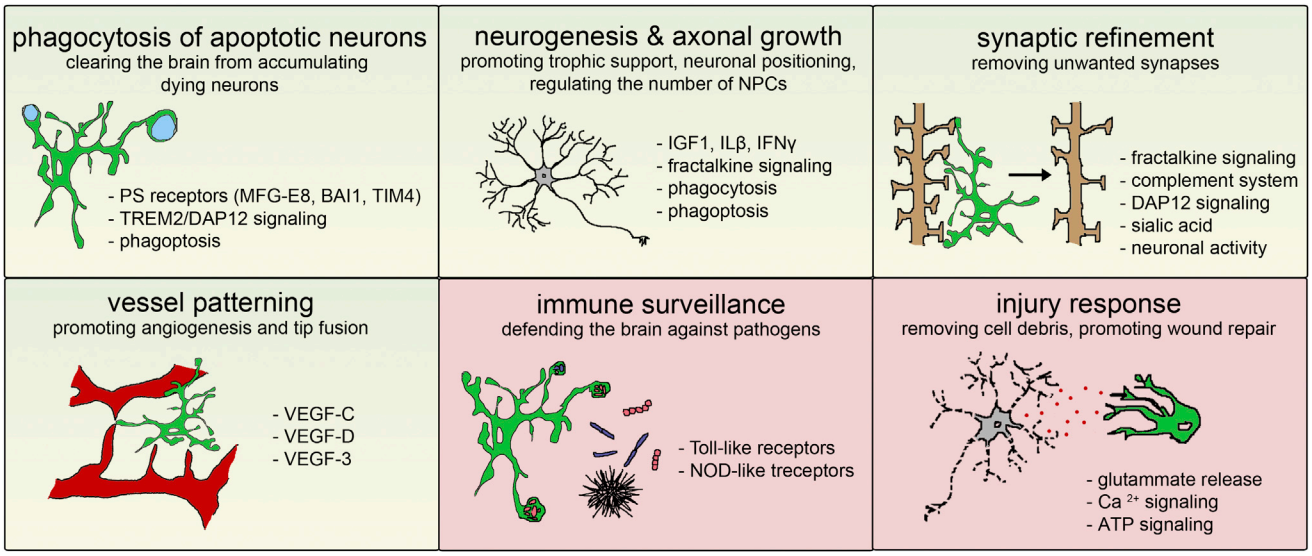

Figure 3. Multitasking Microglia
Schematic representation of microglial roles in the developing brain. Yellow and pink boxes indicate microglial roles during developmental stages and general immune-related functions, respectively.

entry (Mildner et al., 2007; Kierdorf et al., 2013b). Notably, Alzheimer's disease (AD) patients, for example, often present blood-brain barrier defects (Algotsson and Winblad, 2007; Bowman et al., 2007), suggesting that that circulating monocytes might indeed be present in the brain also, without the application of conditioning methods. In this context, it is interesting to consider that myeloid cells might differ in terms of β-amyloid plaque engulfment. In particular, microglia are thought to be poor phagocytes for β-amyloid engulfment and to promote AD pathology by releasing proinflammatory cytokines (Yan et al., 1996; Xie et al., 2006; Griciuc et al., 2013).

The Multitasking Microglia

Microglia have long been studied for their involvement in immunity and in many neurological disorders, such as AD and Parkinson's disease (for a review, see Streit et al., 2004; Lehnardt, 2010). However, there is mounting evidence for important roles of these cells in the context of brain development and in the establishment of neuronal connectivity (Figure 3).

Clearance of Apoptotic Cells within the CNS

Brain development is a very wasteful process characterized by extensive neuronal apoptosis. Indeed, programmed cell death in the CNS is key for establishing the brain cytoarchitecture (Oppenheim, 1991; de la Rosa and de Pablo, 2000; Cole and Ross, 2001; Kuida et al., 1998). Microglia play a central role in removing apoptotic neurons, both during early CNS development and in the adult (Perry et al., 1985; Ashwell, 1990; Calderó et al., 2009; Sierra et al., 2010). Recent work has shown that microglia have a high phagocytic index and that this remains constant not only during early adulthood but also in neuroinflammatory conditions (Sierra et al., 2010). Although the importance of microglial engulfing activity is broadly recognized (Neumann et al., 2009), factors involved in this process remain largely unknown. Apoptotic cells accumulating in the brain are likely to expose so-called "eat-me" signals that allow phagocytic cells to selectively recognize and remove these cells from the rest of the tissue. The best characterized is phosphatidilserine (PS), which is flipped out and exposed to the external surface of the cells and recognized by specialized phagocytic receptors and adaptor molecules. It has been shown that the milk fat globule epidermal growth factor 8 (MFG-E8), exposed on the surface of microglia, directly binds PS (Hanayama et al., 2002) and that its level of expression affects the phagocytic behavior of microglia in vitro, suggesting a role for MFG-E8 in microglial recognition and clearance of dying cells (Liu et al., 2013). Recently, live imaging of the zebrafish embryonic brain has allowed neuronal cell death progression and microglial engulfment to be studied in vivo. This analysis has shown that microglia engulf apoptotic neurons by extending branches toward dying cells. At the tip of these cellular extensions, large phagocytic cups are formed and stretch progressively over the dead cell body. By taking a reverse genetic approach, it has been shown that BAI1 and TIM4, two PS receptors expressed on microglia, work together to mediate apoptotic body removal. In particular, BAI1 promotes phagosome formation, whereas TIM4 stabilizes these structures, most likely by allowing actin polymerization around them (Mazaheri et al., 2014). It is also of great interest to compare microglial phagocytosis to that of other macrophages. This has been done in the context of spinal cord injuries (SCI). Here, by labeling differently microglia and infiltrating macrophages, the authors have shown that microglia contact damaged axons 24 hr after injury, while infiltrating macrophages arrive later and interact mostly with degenerating axons (Greenhalgh and David, 2014). Notably, infiltrating macrophages contain more phagocytic material than microglia, and this is due to the fact that these cells process ingested material much more effectively (Greenhalgh and David, 2014; for a review on the topic, see David and Kroner, 2011). In this context, it has been shown that in zebrafish, the v0-ATPa1 subunit promotes digestion of the engulfed material by mediating vesicular fusion in microglia (Peri and Nüsslein-Volhard, 2008).

One remarkable aspect in apoptotic cell clearance in the brain is the need for long-range recognition of targets, as they are invariably surrounded by many viable neurons and important cell-cell connections. In this context, microglia might be forewarned by chemotactic signals. Signals such as ATP (Davalos et al., 2005) and lysophosphatidylcholine (Lauber et al., 2003) have been shown to attract macrophages and could also work in vivo to guide microglia toward apoptotic neurons (for a review, see Hochreiter-Hufford and Ravichandran, 2013; Chen et al., 2014). Furthermore, the chemokine fractalkine (CX3CL1) has been shown to act as a "find-me" signal released by apoptotic neurons. Experiments inducing neuronal apoptosis with ethanol have shown that soluble CX3CL1 is present in the extracellular medium (Sokolowski, 2014). This promotes the attraction of wild-type microglia but not *Cx3cr1* knockout microglia. Although several signals that attract microglia and macrophages toward apoptotic targets have been identified, it remains to be established how they are transmitted across the brain and how they guide microglia with high precision. Unfortunately, in most contexts, the spatial and temporal dynamics of these cues are not known because of the difficulty in visualizing these stimuli within intact tissues. The development of quantitative imaging probes that allow the dynamics of signaling to be monitored within the intact brain has provided insights into the spatiotemporal control of microglial activation in response to neuronal cell death. By combining targeted laser neuronal ablations with $Ca2^{+}$ reporters in zebrafish, it has been possible to show that graded $Ca2^{+}$ waves define the range within which microglia become targeted to the damaged neurons. Preventing $Ca2^{+}$ signaling is sufficient to suppress microglial targeting, whereas ectopic induction of $Ca2^{+}$ signaling mimics the attraction of microglia to neuronal injury (Sieger et al., 2012). These Ca^{2+} ramps, in turn, lead to the establishment of an ATP gradient that guide microglia via the activation of the P2y12 receptor (Sieger et al., 2012). It is interesting that this receptor has also been shown to guide mouse microglia toward brain injuries (Haynes et al., 2006), indicating that there is potentially a high degree of conservation among the molecular and cellular machineries that regulate microglial responses to cell death both in fish and mouse.

In addition to the removal of dying neurons, microglia might also actively promote neuronal cell death through a process known as "primary phagocytosis" or "phagoptosis." In this context, microglia have been suggested to be responsible for the execution of Punkinje cells in the developing mouse cerebellum and in hippocampal neurons by producing reactive oxygen species, such as superoxide anions, upon activation of the integrin CD11b and the immunoreceptor DAP12. (Marín-Teva et al., 2004; Wakselman et al., 2008). These same factors are also used by neutrophils to kill invading pathogens, indicating that the innate immunity machinery is also utilized to promote neuronal cell death. Similarly, microglial activation using lipopolysaccharide (LPS) stimulates release of reactive oxygen species. This induces reversible PS exposure on viable neurons, leading to engulfment (Neher et al., 2011; Fricker et al., 2012b). In this context, blocking phagocytosis increases neuronal survival (Neher et al., 2011; Fricker et al., 2012a). Finally, in vitro studies have suggested that release of tumor necrosis factor alpha (TNF-α) by activated microglia could promote the microglial-mediated neuronal uptake and phagoptosis (Neniskyte et al., 2014). A similar role for TNF-α secreted by macrophage had already been observed in the mouse spinal cord during embryonic development (Sedel et al., 2004). All together, these studies provide evidence for a new role of microglia in promoting neuronal cell death. This process might be particularly relevant in the context of neurodegenerative diseases, such as AD or Parkinson's disease, where microglia have been shown to have detrimental effects (for a review, see Brown and Neher, 2014). It remains to be established if this occurs also under physiological conditions and if it impacts on brain development.

Supporting Neurogenesis and Brain Wiring

The role of microglia in neurogenesis has mainly been addressed under pathological conditions; for example, during brain inflammation (Ekdahl et al., 2009). However, accumulating evidence has also highlighted the importance of microglia and microglial-mediated production of neurotrophic factors in modulating neurogenesis during embryogenesis and adulthood. In particular, Ueno and co-workers found that these cells promote both neuronal survival and axonal growth by providing insulin-like growth factor 1 (IGF-1) (Ueno et al., 2013). Indeed, in vitro and in vivo approaches have shown that postnatal microglia secrete IGF-1 and that interfering with the IGF-1 signaling pathway results in the accumulation of apoptotic neurons in layer V of the neocortex, where most subcerebral projecting neurons are located. However, in the subventricular zone, recent data point to IL-1β and interferon gamma (IFNγ), rather than IGF-1, as the main signals for microglial-mediated neurogenesis (Shigemoto-Mogami et al., 2014). Microglia have also been shown to promote wiring in the forebrain. Squarzoni et al. (2014) have observed that, at E14.5, microglia accumulate preferentially in specific hotspots in close association with dopaminergic axonal tracts and neocortical interneurons. In the absence of microglia, dopaminergic forebrain axons extend from the forebrain to a more dorsal domain. It is interesting that, upon microglial activation, these extensions are significantly reduced. Neurogenesis occurs also in adults, even though this process is subject to a clear age-dependent decline (Rao et al., 2006). Here, lack of CX3CR1 induces upregulation of different cytokines that have been shown to be either beneficial or detrimental for neurogenesis depending on the brain area (Battista et al., 2006; Bachstetter et al., 2011). Microglia have also been proposed to impact on the number of neuronal precursors as a result of their activation state. Cunningham et al. (2013) have found, for example, that the microglial activation state correlates with the number of neuronal precursor cells (NPCs) and, in particular, that in the presence of activated microglia, there are fewer NPCs. It is interesting that microglia also affect NPC fate in in vitro spinal cord transplantation experiments. Specifically, coculture of NPCs with organotypic spinal cord slices revealed that microglia undergo activation, impairing NPC survival and promoting glial rather than neuronal differentiation (Liu et al., 2013). In contrast to these observations, however, activated microglia in young adult mice have been shown to not directly alter the number of NPCs in the hippocampus but to simply engulf apoptotic neurons (Sierra et al., 2010). Thus, while there is evidence for a role of microglia in modulating neuronal cell number and wiring of the brain, the role of microglia in these processes might not be univocal, but it might vary depending on the biological context and on the molecular factors involved.

Synaptic Refinement

Undoubtedly, one of the most intriguing roles of microglia in the brain is synaptic pruning. This takes place in the mouse during early and late postnatal stages for the remodeling of neuronal connections (for a review, see Holtmaat and Svoboda, 2009). Microglia are known to establish transient connections with neuronal synapses (Wake et al., 2009), and their pruning activity has been well documented in the cerebral cortex, hippocampus, and thalamus of postnatal mouse brains, where inclusions of synaptic elements are often found within the phagocytic compartments (Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012). Paolicelli et al. (2011) have documented microglial pruning during the first 2 postnatal weeks of mouse development. In this context, data from single-cell recordings of spontaneous and miniature excitatory postsynaptic currents revealed that a lack of pruning results in increased connectivity and redundant afferent synaptic inputs, causing impaired motor learning, associative memory, and spatial memory (Paolicelli et al., 2011; Rogers et al., 2011).

Recently, the complement system, a proteolytic cascade that is central in fighting infection, has been shown to play an unexpected role in brain wiring and synaptic pruning. It is interesting that in situ hybridization and gene-profiling analysis have shown that many components of this system are actually expressed by astrocytes and microglia in the healthy brain (Stevens et al., 2007; Cahoy et al., 2008; Woodruff et al., 2010; Veerhuis et al., 2011). In particular, the C1q ligand, known for biding antibodies and for triggering complement activation, localizes with synapses in the retina, and in C1q and C3 receptor knockout mice, there are significant defects in the retinogeniculate (RGC) synaptic refinement (Stevens et al., 2007). Despite the clear support for involvement of the complement cascade in synaptic pruning, the molecular mechanism underlying its control is still unclear. Sialic acid is usually exposed on the outer membrane of neurons and might constitute a link between microglial synaptic refinement and the complement system. Indeed, under in vitro desialylation conditions, neuronal surfaces are marked by C1q, which binds to oliogosaccarides or protein deprived from their sialic acid cap. In this way, C1q-tagged neurons are recognized by the microglia and removed via phagocytosis (Linnartz et al., 2012).

Notably, it has been shown that blocking neuronal activity results in the phagocytosis of "weaker," less active inputs, suggesting that microglia are able to somehow sense synaptic activity (Schafer et al., 2012). Furthermore, microglia actively contribute to the experience-dependent refinement of the synaptic circuits in the healthy brain (Tremblay et al., 2010). Indeed, during light deprivation, which is known to increase dendritic spine motility and turnover, microglial cells undergo morphological changes, showing higher phagocytic activity and increased accumulation of synaptic inclusions (Tremblay et al., 2010). It remains to be established if microglia monitor and interact with each synapse. Indeed, live recordings in mouse have shown that microglial cells continuously scan the brain parenchyma with their motile processes (Nimmerjahn et al., 2005), while in zebrafish, neuronal excitatory activity has been shown to promote direct contact between microglia and neurons (Li et al., 2012). In particular, live imaging has shown that microglia contact neurons characterized by high spontaneous $Ca2^+$ activity. These interactions, in turn, reduce $Ca2^+$ levels in these cells. Thus, neuronal activity is likely to provide directionality to microglial processes, helping them to localize their targets.

Live imaging approaches are crucial tools for understanding the dynamic interaction between microglia and neuronal cells. However, as microglia are powerful sensors of the brain health status, it is important to avoid damage due to sample preparation and photodamage caused by extended imaging. In this regard, new imaging technologies that combine fast scanning with low phototoxicity, such as light sheet microscopy (Huisken et al., 2004; Keller et al., 2008), will play an increasingly important role for studying these sensitive cells.

Conclusions

Work on microglia has shown that these cells perform key glial functions and that they are involved in circuit refinement, neurogenesis, and neuronal growth. Thus, it is clear that these cells are not just simple sentinels that survey the brain but are active multitasking specialists during development, adulthood, and senescence. Indeed, several studies have shown that a lack of microglia has detrimental effects on proper brain functionality, with consequent severe behavioral and learning deficits. Supporting evidence comes from mice lacking functional Hoxb8, where transplantation of wild-type monocytes and microglial engraftment into irradiated mutant mice is sufficient to rescue the compulsive grooming behavior that these animals exhibit (Chen et al., 2010). Also, microglial ablation in mice leads to deficits in learning tasks and motor-learning-dependent synapse formation that can be recapitulated by genetically depleting microglia expressing brain-derived neurotrophic factor (BDNF) (Parkhurst et al., 2013). It is interesting that microglia share some of their tasks with astrocytes, a cell type also involved in synapse elimination and apoptotic removal (Cahoy et al., 2008; Chung et al., 2013; Tasdemir-Yilmaz and Freeman, 2014). This redundancy highlights the importance of these processes, but it still remains to be established how these two cell types share these tasks. The role of microglia as scavengers has been recently reevaluated. Indeed, in the context of Rett syndrome, an X-linked autism spectrum disorder characterized by mutation of the *MECP2* gene, microglia have been shown to have deficits in phagocytosis; as a consequence, $Mecp2^{-/-}$ neurons accumulate and contribute to disease progression. Notably, engraftment of wild-type microglia upon BM transplantation in postnatal $Mecp2^{-/y}$ male mice arrests the pathology (Derecki et al., 2012). This arrest can be prevented by using annexin V to block phagocytosis in conditional knockout mice with wild-type microglia and $Mecp2^{-/-}$ neurons, suggesting that engulfment of apoptotic neurons and/or of cellular debris by microglia is central for blocking disease progression. One remarkable finding of this work is that boosting microglial phagocytosis might reduce the devastating impact that neuronal cell death has in the context of many neurodegenerative diseases, thus opening new avenues in therapy development.

Thus, as new and important roles emerge for microglia, both under physiological and pathological conditions, it is necessary to achieve a true mechanistic understanding of how microglia work and interact with other cell types in the brain. We believe that this will require asking questions about how microglia sense brain health status and how these cells makes collective decisions to establish how they subdivide the task of scanning and

how they coordinate appropriate responses. Indeed, microglia-microglia communication has yet to be addressed by in-depth investigation. Finally, it is crucial to know how microglial responses are tailored to the many different stimuli that trigger them. Although an important step, the identification of molecules involved in these processes will only be the beginning to understanding these complex, multicellular questions. Ultimately, we need to understand how these molecules and the responses to them are controlled, both in space and time, to regulate the behavior of the entire system of microglia. Such questions will surely keep these multitasking cells in the spotlight for a very long time.

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